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## ABC transporters in the blood-brain barrier – Relevance in stroke therapy

**Mónika Bollók, Judit Jánossy and Péter Krajcsi**

SOLVO Biotechnology Inc. Central Hungarian Innovations Center, Gyár u. 2  
H-2040 Budaörs, Hungary

### Abstract

*There is a great demand to find effective treatment for stroke patients. Much effort has been made to target medication specifically to the affected brain area. The blood-brain-barrier strictly controls the amount of xenobiotics that can enter the brain in order to protect the fragile and sensitive homeostasis. The penetration of drug molecules through the blood-brain-barrier is restricted by various mechanisms such as tight junctions and transmembrane transporter proteins. Even if a drug gets across the membrane of the brain capillary endothelia, it is liable to be pumped back to the blood stream by efflux*

Correspondence/Reprint request: Dr. Péter Krajcsi, SOLVO Biotechnology Inc., Central Hungarian Innovations Center, Gyár u. 2, H-2040 Budaörs, Hungary. E-mail: krajcsi@solvo.com

*transporters. Transporters may further influence stroke therapy via regulating the bioavailability and pharmacokinetic characteristics of drugs. Co-administration of drugs may result drug-drug interaction which is another aspect that must be considered at therapeutic interventions. This review gives insight into methods to detect transporter-drug interactions and discusses the interface of transporters and stroke.*

## **Abbreviations**

ABC transporter (ATP binding cassette) transporter; ABCB1: Pgp, MDR1; ABCC family MRP multidrug resistance associated protein; ABCG2/BCRP/MXR breast cancer resistance protein/mitoxantrone resistant protein; ADME absorption, distribution, metabolism and elimination; BBB blood brain barrier; BCSF blood-cerebrospinal fluid barrier;

## **Introduction**

The transport of molecules across the blood brain barrier (BBB) is a highly restricted and controlled process. The tight junctions between adjacent cells, lack of capillary fenestration and low pinocytotic activity hamper transport across BBB. The major pathway for compounds to cross the BBB is the transcellular route, which is dependent on their physical-chemical characteristics and interactions with transporter proteins.

Two transporter superfamilies can be distinguished: the ATP-binding cassette (ABC) transporter and the Solute Carrier (SLC) superfamilies. They include transmembrane proteins that translocate their substrates across extra- and intracellular membranes. Members of the SLC superfamily, the so called uptake transporters assist the entry of compounds into the cells, whereas proteins of the ABC superfamily use ATP to actively pump out compounds from the cells. A wide array of substrates including xenobiotics such as drugs, nutrients, and toxins and their metabolites, as well as endogenous substances such as bile acids, peptides, steroids, ions, and phospholipids are translocated by transporters.

This review focuses on the relevance of ABC transporters at the blood-brain-barrier with special emphasis on their potential influence on stroke treatment. After a brief description on the importance of transporters in the brain and how transporter drug interactions can be measured, the consequences of these interactions will be discussed highlighting the recent advances to overcome their influence. Transporters modulate the pharmacokinetic properties of drugs, including the targeting of neuroprotective agents used for stroke therapy. Transporters may have an impact on stroke treatment i) by influencing the pharmacokinetic profile of drugs and thereby be responsible for adverse side effects and ii) transporters may inhibit drugs from reaching their

target site. On one hand transporters influence stroke therapy, yet on the other hand the pathological processes also modulate the activity and expression of transporters. The review gives a short overview of factors that should be considered when developing new therapeutic interventions for stroke therapy.

## 1. Importance of transporters in the brain

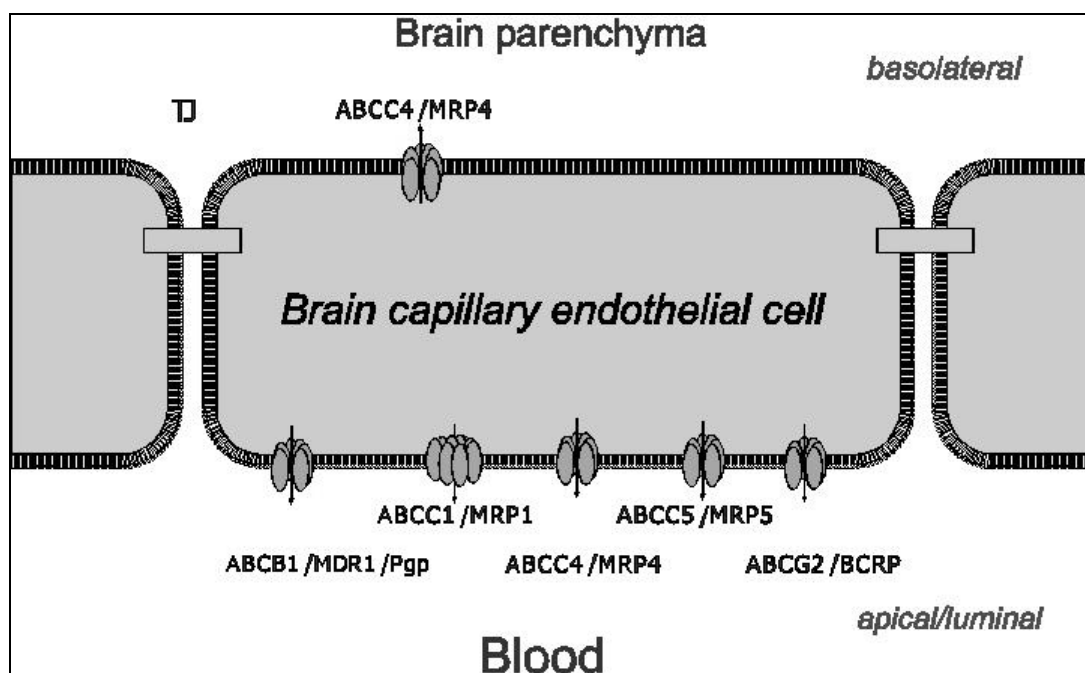
Transporters are abundantly expressed in the brain; they are present in the endothelial cells of the BBB, the epithelial cells of the blood-cerebrospinal fluid (BCSF) barrier and also in brain parenchyma cells [1]. Drug uptake into the brain highly depends on the efflux transporters expressed at the BBB and BCSFB. The BCSF is more permeable to drugs compared to the BBB and therefore may have a distinguished role in the exchange of xenobiotics between blood and brain. The drug concentration in CSF is a common surrogate measure for critical unbound brain concentration of the drug in clinical drug development [2]. However, it must be noted that the scientific society is divided on the relevance of data generated on choroid plexus. In addition, the transporter expression, localization and function are ambiguously characterized in the BCSF. Therefore, our review will only focus on studies utilizing various BBB models.

In the BBB the endothelial cells connected by tight junctions completely seal paracellular transport and form a continuous capillary structure. Central nervous system (CNS) drugs entering the brain must therefore cross the endothelial cells. Depending on the lipophilicity of the drugs they can either readily penetrate through the BBB membranes or require carrier-mediated transport to enter the cells. But, xenobiotics and endobiotics that cross the apical membrane of the epithelia may still be pumped back to the blood stream by ABC transporters. Efflux transporters in the brain capillary are mainly present on the luminal (apical or blood side) side of the endothelial cells (see Fig.1). The highly polarized structure of the epithelia with distinct transporter protein distribution on the apical (luminal) and the basolateral (abluminal) sides enable the vectorial transport. This way the uptake of xenobiotics in the brain is hindered. Though transporters protect the brain from toxic compounds, they also deter central nervous system drugs to reach their target site, which may be a reason for therapeutic inefficacy [3]. Despite the intense research to identify and localize ABC transporters in the BBB solid data exists only on ABCB1, ABCG2, ABCC1, ABCC4 and ABCC5.

ABCB1 also known as P-glycoprotein (P-gp) or multidrug resistance transporter (MDR1) was the first ABC transporter identified in the 1970's. ABCB1 is known to mediate multidrug resistance, displays a protective function as well as plays a crucial role in determining the ADME properties of

drugs. It is present in all major barriers in the epithelial and endothelial cells in the apical/luminal membranes. It has a broad substrate specificity, transports hydrophobic compounds and unconjugated cationic substances. ABCB1 plays a key role in limiting the brain penetration of several drugs, among others antidepressants [4], amyotrophic lateral sclerosis drugs [5]. ABCB1 is encoded in human by the ABCB1 gene while rodents express two isoforms. *Abcb1a* is found in brain microvessels, whereas *Abcb1b* has been detected in astrocytes [6]. In rodents, *Abcb1a* have been found to contribute to the transport of drugs from the brain into the blood [7].

ABCG2, also known as breast cancer resistance protein (BCRP), and mitoxantrone resistant protein (MXR) was simultaneously recognized by three groups. Though its major physiological role is still uncertain it functions as a drug transporter with extremely wide substrate specificity. A number of reviews have been published recently dealing with the role of the transporter in drug ADME, in hypoxia, in drug and environmental toxin elimination, and at the blood-brain barrier [8-11]. ABCG2 is most abundant in the placenta, followed by the liver and the small intestine. It is highly expressed in the luminal membrane of the capillary endothelial cells of the blood-brain barrier too. Its frequent expression in highly dividing cells and stem cells [12] imply that ABCG2 is related to growth and cellular division. Thus, ABCG2 may play a role in the revascularization and regeneration processes following stroke.



**Figure 1.** Efflux transporters expressed in the BBB.

Members of the ABCs/MRPs multidrug resistance-associated protein (MRP) family transport conjugated anionic substances such as acidic xenobiotics, endobiotics or phase II conjugates. Some of the ABCs also transport hydrophobic compounds in a cotransport mechanism with GSH. ABCs are also believed to add to the resistance of the brain to drugs [13]. The expression of ABCC1, ABCC2, ABCC3, ABCC4 and ABCC5 mRNA was detected in human brain, but only protein of ABCC1, ABCC4 and ABCC5 were localized at luminal side of brain capillary endothelial cells. ABCC4 and ABCC5 are also expressed in astrocytes [14]. ABCC5 has been besides capillary endothelial cells and astrocytes in pyramidal neurons also, and has a likely role in cell signalling *via* transport of endogenous signaling molecules [14, 15]. There are contradicting data on the presence of other ABCs such as ABCC2, ABCC3 and ABCC6 in the brain probably as a consequence of the different detection methods [14].

The most important human efflux transporters, localization, substrates and examples of their function are presented in Table 1.

**Table 1.** Most important efflux transporters expressed in human brain [13, 14, 28-33].

<i>ABC / Efflux transporters in the brain</i>				
<b>Transporter</b>	<b>Rodent genes</b>	<b>Localisation</b>	<b>Substrates</b>	<b>Function</b>
P-gp /ABCB1/ MDR1	mMdr1a mMdr1b rMdr1a rMdr1b	Epithelial, Endothelial cells	Hydrophobic, unconjugated cationic substances	P-gp limits the brain penetration of oseltamivir, an anti-influenza drug [16]. It also limits penetration of 2 <sup>nd</sup> generation antihistamines yielding a favourable safety profile to these drugs [17-19].
MRP1/ABCC1	mMrp1 rMrp1	Ubiquitous, endothelia in the brain	Endogenous anionic glutathione and glucuronate conjugates	overexpression of MRP1 in the brain of epileptic patients is one of the reason of the resistance to anti-epileptic drugs[20] ABCC1 protects the brain from etoposide [21]
MRP4/ABCC4	Rat mMrp4	Many tissues, Endothelial cells and astrocytes in the brain	Cyclic purine nucleotides, nucleotid analogs, phase II sulphate conjugates	Transports prostaglandins across the BBB, and BCSF, thereby partake in the fever response of the brain [22] It also plays a role in the leukotrien mediated inflammation in the brain [23].
MRP5/ABCC5	rMrp5 mMrp5	Many tissues, Endothelial cells, astrocytes and pyramidal neurons in the brain	Cyclic purine nucleotides and nucleotid analogs	MRP5 (together with MRP4) restricts the permeability of different antiretroviral drugs in the brain[24]. ABCC5 expression was upregulated in a stroke model in neurons [15]
BCRP/ABCG2/ MXR/ABCP	mBcrp1	Placenta, liver, small intestine, endothelium (in the brain)	Amphipatic drugs, DHEAS; Folates; Porphyrines; Riboflavin; Sulfated Estrogens; Steroids	ABCG2 limits the distribution of phytoestrogens into the brain [25]. Together with ABCB1 responsible for limiting the brain penetration of topotecan and other anticancer drugs [26, 27]

## **2. Models / methods used to study transporters in the BBB**

### **2.1 *In vitro* models / methods**

#### **2.1.1 Membrane models and assays**

Membrane preparations from recombinant baculovirus infected insect cells, and from selected or transfected mammalian cell lines containing high levels of a transporter are widely used to detect interactions of compounds with different ABC transporters in a high throughput manner [34-37]. Membrane preparations contain a mixture of “inside-out” and “rightside-out” oriented intact membrane vesicles. Two distinct types of membrane assays are utilized: the ATPase and the vesicular transport assay.

##### **a. ATPase assay**

ABC transporters mediate transport against a concentration gradient using ATP as energy source. In the ATPase assays the rate of liberation of inorganic phosphate (Pi) yielded from ATP hydrolysis is measured. This correlates well with the activity of the transporter and is easy to detect. With the ATPase assay two different protocols are used to characterize the interactions: the (i) ATPase activation and the (ii) ATPase inhibition assays. (i) In the activation assay stimulation of the basal ATPase activity of the transporter is measured. A test compound that significantly stimulates the basal ATPase activity is considered a likely substrate of the transporter. (ii) In inhibition assays the ATPase activity of the transporter is activated by a known substrate. In the presence of an interacting test compound the inhibitory effect of the compound can be evaluated. This assay type is useful to identify inhibitors and slowly transported compounds that do not stimulate the basal ATPase activity.

##### **b. Vesicular transport assay**

The vesicular transport assay (VT) consists of the direct or indirect measurement of ATP dependent transport of compounds into inside-out membrane vesicles. In the direct assay the transport of the test compound is measured whereas the translocation of a reporter substrate is detected in the indirect assay. The transport can be detected by LC/MS or LC/MS/MS directly or in case of fluorescent or radioactive compounds by spectrofluorimeters. In the indirect version of the assay usually fluorescent or radioactive reporter substrates are utilized. This simple, rapid and relatively cheap method allows for HT screening of transporter compound interactions. Its disadvantage is that it can not distinguish between the inhibitor and substrate nature of a compound.

### 2.1.2 Cellular models and assays

The first viable brain endothelial cells maintained in cell cultures were prepared by Panula and co-workers in 1978. Cell models to measure drug transport with primary and immortalized brain endothelial cells and immortalized cells from peripheral tissues were developed later. Different types of transport assays can be performed depending on the properties of the cells culture (see below). Cells derived from brain capillary endothelia closely mimic the physiological characteristics of their source, *e.g.* the most important transporters, biomarkers and enzymes are expressed and functioning. These cell lines express a wide range of transporters that facilitate the uptake and efflux of compounds. The transporter expression profile may vary with passage number and seeding leading to reduced reproducibility. A major drawback of using these cells is that they rarely form adequately tight monolayer that is required to measure vectorial transport. Presently, none of the human brain endothelial cells are known to form tight monolayers in culture in contrast to rodent, bovine and porcine brain endothelial cells [33, 38]. Several methods increase the tightness of the monolayer such as using astrocyte-conditioned medium; coculture with astrocytes; coculture containing pericytes and astrocytes; or supplement the medium with hydrocortisone or phosphodiesterase inhibitors and cAMP analogues[39]. To date, one of the best available *in vitro* human brain endothelial cell model is hCMEC/D3, a transduced cell line with suitable characteristics for uptake transport measurements [39]. Unfortunately, due to its large paracellular permeability it's not applicable for vectorial transport. To evaluate the individual contribution of specific transporters that are expressed in the BBB transfected cell lines from peripheral tissues are commonly utilized (*e.g.* LLC-PK1, MDCKII porcine and canine kidney respectively) [25, 40-44]. These cell lines are suitable for monolayer vectorial transport assays and uptake assays. The advantage of these cells is that the endogenous transporters are downregulated, whereas they highly express the transfected transporter. Recently double and multiple transfected cell lines have also been generated where both uptake and efflux transporters were transfected [45].

#### a. Dye efflux assays

The fluorescent dye assays are cellular assays that provide indirect information on interactions between an ABC transporter and a test drug using a reporter substrate. The accumulation of a known reporter substrate is measured in the presence and absence of the test compound. The accumulation is generally compared to the accumulation of the reporter in the presence of a known inhibitor. The calcein assay and rhodamine 123 transport assays are used commonly for the detection of ABCB1 and ABCC1 transporter interaction s[46] whereas for ABCG2 the Hoechst 33342 assay [47] and the

pheophorbide A assay [48] is the most suitable. For ABCC4 and ABCC5 no specific dyes have been identified so far. This method provides data with functional relevance and is specifically applicable to detect potential drug-drug interactions in a high throughput manner. The disadvantage is that it can give false negative results for low permeability compounds that can not cross the cell membranes. Another drawback is that we can not differentiate a substrate from an inhibitor.

### **b. Uptake assay**

In the uptake assay cells overexpressing the efflux transporter are incubated with the substrate in the presence and absence of inhibitors. Substrate uptake is given as the cell-to-medium concentration ratio [49, 50]. If a drug is a substrate of an efflux transporter the uptake of the compound is increased in the presence of the inhibitor; and the accumulation of the compound is decreased in cells highly expressing the transporter compared to that measured with parental cells. The amount of the test compound is detected analytically e.g. by LC/MS, or can be labeled by a fluorescent or radioactive tag and detected accordingly. Alternatively, it can be performed as an indirect assay using a reporter substrate (*e.g.* see dye efflux assay).

### **c. Monolayer efflux assay**

In the monolayer assay the vectorial transport of compounds is measured using tight monolayers expressing transporters [51]. Markers for paracellular, transcellular, and transporter-dependent efflux must be included in the experimental design. Transporter specificity is secured either by the application of specific inhibitors or by comparing the test compound transport rate of transfected to parental cells. Between the apical (A) and basolateral (B) side both unidirectional and bidirectional assays are performed. If a compound is the substrate of an apically located efflux transporter its permeability is greater in the B-to-A direction than in the A-to-B direction. The ratio of the permeabilities is called the efflux ratio. The inhibition of an apical transporter increases the A-to-B transfer of substrates and decreases the permeability in the opposite direction (B-to-A). The efflux ratio approaches 1 in the presence of a specific inhibitor.

## **2.2 Tissue culture models**

### **2.2.1 Isolated brain capillaries**

Isolated brain capillaries were one of the first models used to study BBB. They are composed of endothelial cells, pericytes ensheathed by the basement membrane, and to which astrocytic processes may also be attached [52]. Brain capillaries were isolated from different species including rat [53], and pig [54]. Experimental studies indicated the presence of P-gp activity and the members

of the MRP subfamily [54]. Although this model contains all the main components and metabolic activities of the BBB, the preparation procedure can cause changes in the activity, expression and subcellular localization of transporters, and may result in the decrease of ATP [1].

## **2.3 In situ/ in vivo models**

### **2.3.1 Knockout mice and natural transporter defective mutants**

Several transporter knockout mice models [28, 51, 55] have been used to gain better insight into the role of transporters at the blood – brain barrier. One of the drawbacks of these in vivo models is that compensatory changes may occur [56]. *Abcb1a* (-/-) knockout mice are commonly used to study the function of ABCB1 in the BBB [56, 57] despite the availability of ABCB1 deficient double knock-out mice (*Abcb1a*(-/-)/*1b*(-/-) as in the BBB the *mdr1b* isoform of ABCB1 is expressed insignificantly. The protective role of ABCB1 from the neurotoxic effect of avermectins was demonstrated with CF-1 mice, an inherently ABCB1 defective mutant mice strain [58]. Imatinib mesylate (Gleevec) was shown to display *Bcrp1* dependent brain uptake in *Abcg2*(-/-) and wild type mice model [42]. Interestingly, only non-*mdr1* *Bcrp* substrates with low to intermediate passive permeability show *Abcg2*-limited brain penetration in mice [25]. The involvement of *Abcc1* in the efflux of estradiol-17 $\beta$ -glucuronide [28] and *Abcc4* in the protection of brain from topotecan [59] was also reported using in vivo models.

### **2.3.2 Brain Efflux Index (BEI) method**

The Brain Efflux Index (BEI) is widely used to study the distribution of a compound between the brain and the blood [60].

$BEI (\%) = (\text{amount of test substrate effluxed at the BBB} / \text{amount of test substrate injected into the brain}) \times 100$ .

The test substrate and a BBB impermeable reference compound are simultaneously injected into the brain and the efflux of both compounds is monitored. This method is useful to determine the BBB clearance of the compound of interest [61].

### **2.3.3 In situ brain perfusion**

In this method artificial perfusion fluid containing the compound of interest is directly infused into the carotid artery and the brain drug concentration is determined. Thus, the brain distribution at a controlled concentration of the drug can be monitored without taking into account the influence of metabolism in the peripheral organs. This allows a simple kinetic study especially for radiolabeled compounds and is commonly performed with rodent species [56, 62].

### 2.3.4 In vivo brain microdialysis

The implication of in vivo brain microdialysis techniques in preclinical stroke research is described in detail in Chapter 14. Here we mention only one aspect; the role of microdialysis in pharmacokinetic studies of drugs and drug candidates (potentially useful in the treatment of stroke). In short, a probe is placed into a specific brain region to study the free drug concentration in the extra cellular fluid after peripheral administration of a drug. The distribution of a drug in the brain can be determined in anesthetized and also in freely moving animals. The application of specific inhibitors or knockout mutant animals allows for clarification of the role specific transporters have on drug penetration [1].

### 2.3.5 Imaging techniques

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are used to study in vivo BBB transporter function using radiopharmaceuticals in combination with modulators [1]. These noninvasive techniques are useful tools in drug development and to identify drug-drug interactions. A number of labeled P-gp substrates are used, most frequently [11C]-verapamil [63], but also [11C]-colchicine and [11C]-daunorubicin have been applied [64]. The activity of MRP1 is also monitored by PET using N-[11C]-acetyl-leukotriene E4 as substrate [65]. P-gp, MRP1 and MRP2 substrates 99mTechnetium-labeled tetrafosmin and sestamibi are extensively used for nuclear medicine imaging, especially in brain tumor clinical studies [65, 66].

## 3. The pharmacology of stroke

Stroke is a cerebrovascular accident (CVA) caused by an interruption in the blood supply affecting a part or all of the brain leading to neuronal damage and loss. Two subtypes of stroke are distinguished: hemorrhagic, when a blood vessel bursts in the brain; and the more frequent ischemic type is due to an occlusion in the blood vessel that inhibits blood flow, thereby causing reduction of oxygen and nutrient supply in the affected area. Hypoxic and hypoglycemic conditions trigger a number of cellular and molecular cascades, also referred to as ischemic cascade that promote the progression of the infarcted area as well as induce protective mechanisms [67].

Among pharmacological therapies of ischemic stroke treatments directed to recanalize the occluded artery, such as thrombolysis, and/or use of neuroprotective drugs to restrict the size of the affected area are being developed. Presently, thrombolytic therapy using the tissue-type plasminogen activator (t-PA) is the only primary treatment of acute stroke. However, the narrow therapeutic window, the risk of hemorrhage, and the need of a

computed tomography scan limit its usage. Other pharmacologic treatments comprise antithrombotics, such as anticoagulants, like warfarin or antiplatelet agents, like aspirin. The factors that increase the risks of recurrence, like high blood pressure or hyperlipidemia are usually treated also. Search for safe and efficacious therapies for the treatment of stroke remains a high priority. Besides the development of reperfusion agents, much effort is directed to develop alternative strategies that act at various sites of the ischaemic cascade [67].

Unfortunately most novel reperfusion techniques and neuroprotective strategies, lack efficacy or have unacceptable toxicity. For example, ximelagatan, a direct thrombin inhibitor and a possible replacement of warfarin turned out to be hepatotoxic in Phase III trials and had to be withdrawn. The high safety and efficacy parameters in animal models of a nitron-derived free radical scavenger, NXY-059, raised high hopes, but disappointingly, no beneficial effect over placebo was found in the second Phase III trial [68]. Traxoprodil (CP-101606), a potent glutamate receptor antagonist has also a low efficacy in the treatment of ischemic stroke [69]. There are other new drug candidates in clinical trials such as the calcium chelator, DP-b99; an astrocyte modulating agent, ONO-2506; edaravone, a hydroxyl radical scavenger; and a membrane stabilizer, citicoline [67]. Though they are still in clinical development phases their efficacy is ambiguous. Maybe an alternative strategy will be to give nutritional supplements like polyphenols, or other natural antioxidant compounds to patients [70, 71]. In summary, inspite of the large efforts to develop small molecular drugs for stroke treatment to date there are only limited signs of success, the safety and cost risks of novel drug candidates are still high (as reviewed by [67, 72-74]).

## **4. Role of transporters in the treatment of stroke**

### **4.1 Drug and drug candidate interactions with efflux transporters**

Drugs and drug candidates targeting stroke treatment and/or prevention either a) act on the occlusion formation or b) limit neurological damage following stroke by interfering with the occlusion initiated mechanisms. The latter include alternative strategies targeting various steps of the ischemic cascade like free radical formation, glutamate induced excitotoxicity, calcium overload, etc. The transporter interactions are important as they influence the pharmacokinetic properties of drugs and ultimately may be the cause of drug-drug interactions. Several drugs and drug candidates have been shown to interact with efflux transporters. Since the blood-brain barrier is the most efficient barrier where transporter interactions play the greatest role [75] it is reasonable to assume that blood-brain barrier modulates/inhibits brain penetration of efflux transporter substrate drugs and drug candidates used in

stroke. In this section we will briefly overview the drugs and drug candidates used in stroke therapy that are known to interact with efflux transporters. Drugs and drug candidates were grouped depending whether they a) act on the formation of the occlusion or b) are neuroprotective agents.

#### **a. Drugs and drug candidates acting on the formation of the occlusion**

Most frequently ABCB1-drug interactions are investigated. One example is warfarin, a widely used anticoagulant drug whose hepatic elimination is influenced by ABCB1 activity. A nonsynonymous single nucleotide polymorphisms of ABCB1 was shown to contribute to warfarin sensitivity [76]. There is a number of known warfarin-drug and warfarin-food interactions associated with ABCB1 activity [77-79]. Ximelagatran, an oral direct thrombin inhibitor that was expected to replace warfarin is another ABCB1 substrate. Unfortunately, the risk of hepatotoxicity stopped the development of ximelagatran. Co-administration of melagatran, the active form of ximelagatran with ABCB1 substrates erythromycin or azithromycin [80, 81] increased the bioavailability of melagatran in human subjects. In vivo and in vitro experiments suggested the interactions are the result of ABCB1 inhibition that leads to decreased biliary elimination of melagatran. Dipyridamole is another antithrombotic drug used in conjunction with aspirin to prevent the recurrence of stroke [82] is a known ABCB1, ABCB2 inhibitor [83-85] and ABCG2 substrate and inhibitor [86]. Coadministration of dipyridamole with digoxin can lead to increased digoxin bioavailability [87].

#### **b. Neuroprotective drugs and drug candidates**

To date, most drugs and drug candidates developed to reduce ischemic brain injury failed in clinical trials, they were toxic or lacked efficacy (for reviews see [67, 74, 88]). Ideally, neuroprotective drugs cross the BBB to reach their target site of action without causing intolerable side-effects. Out of the many strategies we discuss drugs and drug candidates known to interact with efflux transporters and which are free radical scavengers or blockers of glutamate induced excitotoxicity.

Free radicals are generated in the brain during ischemic injury and are involved in secondary injury processes. Treatments with anti-oxidant compounds reduce ischemic brain damage in a variety of animal models, but up to date failed translation into the clinical setting. The potent ABCB1 inhibitor [89] tirilazad, a 21-aminosteroid free radical scavenger was intended for stroke treatment. Yet, it was found ineffective in several ischemia animal models and in clinical trials. Edaravone, a free-radical scavenger has been approved by the regulatory authority in Japan for the treatment of stroke patients [74]. Two transporters present in the BBB, ABCC4 and ABCG2 have been suggested to play an important role in the renal elimination of edaravone

and its metabolites [90]. Interestingly, the approval of edaravone was denied by the FDA as it was thought to be inefficacious, while in Japan the post-marketing surveillance reported serious adverse reactions, including renal and hepatic disorders [91]. These findings indicate that transporter interactions may present a danger. However, transporter interactions are unlikely the reason for the disappointing failure of the antioxidant, NXY-059 which was not intended to go through the blood brain barrier. Here emphasis is given to drugs and drug candidates antioxidant natural compounds are treated in section 5.2 discussed.

Glutamate mediated excitotoxicity is another molecular target in cerebral ischemia for neuroprotection is. Preliminary reports indicate that dextromethorphan (DM), an NMDA antagonist is a promising agent to protect against neuronal damage. However, its inadequate penetration through the BBB is of concern. The rapid metabolism of the drug, resulting in low bioavailability was proposed to hamper its therapeutic utility. Co-administration with quinidine reversibly inhibited the first pass elimination and hence increased the bioavailability of DM [92]. Quinidine is a specific inhibitor of CYP2D6 and is believed to be an ABCB1 substrate although there is some conflicting evidence. Contradicting to Arellano and colleagues [93] who used a rat everted gut model other in vivo studies using rat [94] and knock out mice models [95] claim DM to be an ABCB1 substrate. Co-administration of DM with verapamil increased the brain uptake of DM without altering the systemic concentrations [94] and similar findings were obtained using k.o. mice [95]. The entry of rituzole, a glutamate antagonist, to the brain was found to be limited by ABCB1; it was shown to be an ABCB1 substrate [5, 96, 97]. Another blocker of glutamate mediated excitotoxicity, the calcium channel blocker diltiazem is commonly used for the treatment of hypertension and angina pectoris. In a report using a human neuronal cell culture diltiazem protected against excitotoxicity [98]. Diltiazem is a known ABCB1 and cytochrome P450 3A4 inhibitor.

All in all, there's currently a great unmet need for alternative treatment strategies aimed at prevention or rescue of the neurological tissue damage following stroke. However, drug candidates fail to be implemented in the clinical settings largely due to inefficacy or toxicity. The specific role of transporter interactions remains unclear, yet overcoming the BBB, in certain cases *via* bypassing the transporters might be advantageous. Caution should be taken as transporters may influence the ADME properties of drugs and adverse drug interactions may potentially involve transporter based mechanisms also.

## **4.2 Antioxidant natural compounds and their interaction with efflux transporters**

Food ingredients and traditional Chinese medicine contain numerous natural antioxidant compounds. These antioxidants may modulate the

expression pattern and/or function of transporters, and similarly their availability may depend on transporters.

A traditional Chinese medicine, Danshen and its purified constituents, tanshinone IIA and tanshinone IIB derived from the root of *Salvia miltiorrhiza* Bunge are used for the treatment of stroke. The low availability of tanshinones [7, 62] in the brain was suggested to be the result [7, 62] of ABCB1-mediated efflux.

Studies in the literature on natural compounds and transporter interactions are mostly focusing on polyphenols. [7, 62] Rodent studies have demonstrated the beneficial effect of polyphenol containing nutrients such as vitamin E, green tea extract, ginkgo biloba extract containing quercetin, resveratrol and niacin in cerebral ischemia and recirculation brain injury [99]. The neuroprotective effect of resveratrol (3,4',5-trihydroxy-trans-stilbene), a non-flavonoid polyphenol mostly present in grapes has been implicated in several studies [100-102]. Besides their role as antioxidants, polyphenols were found to be good candidates for multidrug resistance (MDR) reversal agents [103]. In a study using immortalized rat brain endothelial cells (RBE4) Youdim and co-workers [44] reported that quercetin and naringenin, another flavonoid present in orange, could cross the BBB model. Yet, quercetin interacted with both ABCB1 and ABCG2, while naringenin *in vitro* acted as an ABCB1 substrate, but not in the *in situ* brain perfusion models probably due to its high permeability. Polyphenols also interact with ABCG2 [44, 104], ABCC1 and ABCC2 transporters [25, 105-107].

By inhibiting the efflux transporters at the blood-brain barrier and other key barriers polyphenols are prone to modulate the effect of simultaneously administered transporter substrate drugs. It is reasonable to suppose that inhibiting the most crucial transporters at the brain could facilitate brain uptake of drugs. Further research is needed to elucidate the plausible contribution of polyphenols in the treatment of stroke, likely as nutritional supplements or in combination therapy.

## **5. Strategies to overcome the transporter barrier**

There are various strategies to improve brain targeted drug delivery for novel therapeutic interventions. Attempts addressing to overcome the BBB among others include viral delivery [108]; receptor-mediated transcytosis [108]; ultrasound-induced hyperthermia (USHT) that site specifically reduce the integrity of the BBB; application of nanoparticles [109, 110]; and modulation of transporters at the BBB.

It is reasonable to assume that circumventing the efflux transporters at the BBB would improve therapeutic response in stroke patients. One way for achieving this goal is the development of therapeutic agents that do not interact with any of the proteins responsible for efflux at the BBB. However,

as efflux transporters possess wide substrate specificity the success of developing such an agent is limited. Another way of targeting brain penetration is the development and co-administration of specific transporter inhibitors to prevent the extrusion of neuroprotective drugs. So far, ABCB1 inhibitors have achieved the greatest attention. Third generation specific ABCB1 inhibitors, zosuquidar (LY335979), tariquidar (XR9576), valsodar (PSC833) and elacridar (GF120918) are mainly in the development phase in various research laboratories; their clinical efficiency are not yet confirmed. The primary reason for their development was to circumvent multidrug resistance in tumor patients. Nonetheless, *in vivo* preclinical data exist on their potential clinical application in neurological therapeutics, like pharmacoresistant epilepsy [111-113], antipsychotic drugs [114], antiretroviral therapy [115, 116], brain tumors [117, 118, 119] and in focal cerebral ischemia [120]. The inhibition of other efflux transporters has also been studied intensively. Besides ABCB1, modulation of ABCG2 and transporters of the ABCC family could also be a target to overcome the transporter barrier and partake in therapy [3, 59, 121]. Different formulation strategies, like the use of certain excipients can also modulate drug disposition and aid drug penetration through the BBB [31, 49].

However, the risk of adverse effects related to transporter inhibition must not be neglected as potential pharmacokinetic interactions may arise, making the development and clinical application of these transporter modulators difficult. Modulation of excretion, bioavailability, high dose toxicity and the considerable overlap of transporter and metabolizing enzymes (CYP P450 family) interacting compounds are examples of such adverse reactions.

An alternative strategy to by-pass the transporters is ultrasound-induced hyperthermia (USHT) that can selectively increase the membrane permeability of the BBB [122]. The advantage of this method is that it can be limited to a small area. In a cancer cell model combination of USHT and transporter inhibition to augment cellular drug uptake was reported to be more effective than any of the treatments alone [123].

Despite the various strategies to selectively overcome the transporter barrier in the brain further investigations are needed to understand and develop more efficient and targeted ways to enable the delivery of pharmaceuticals to the pathological sites.

## **6. Impact of pathology on transporter status in BBB**

Disruption of the BBB occurs in a number of pathological conditions, including Alzheimer's disease, diabetes, inflammatory pain, and stroke [124] characterized by loss of of BBB monolayer integrity, increased paracellular permeability suggesting the disruption of the TJ. In the core region of stroke hypoxic/ischemic and aglycemic insults induce the disruption of the BBB and trigger inflammation. In order to treat stroke it is important to limit the

neurological tissue damage within the penumbra region. Hence, neuroprotective pharmacological therapy focuses on the penumbra region where shortly after the onset of stroke the effects are not yet so explicit.

Under pathophysiological conditions, such as stroke the level and pattern of protein expression may considerably alter. In line with the plausible protective role of transporter proteins their expression was reported to be modified too. However, data on transcriptional changes in the brain following stroke are contradicting and highly depend on the model used. *Abcb1a* expression appeared in neurons following local vasculature destruction at the close proximity of the area affected [125], and in neurons and astrocytes following  $\text{CoCl}_2$  induced hypoxia [126]. Interestingly, *ABCC5* was also detected in surviving neurons of the peri-infarct area suggesting a correlation between their up-regulation and the level of cGMP [126]. In a focal cerebral ischemia model the expression of *Abcb1a* in the ischemic brain compared to non-ischemic brain was significantly augmented in CD31+ endothelial cells, but not in neurons and nor in astrocytes. The transporter protein expression started to decrease after 24 hours of treatment [120]. Inhibition of the transporter potentiated the neuroprotective effect of rifampicin and tacrolimus [120]. Thus, it seems that within 24 hours following stroke the efflux activity of ABCB1 at the affected penumbra area is increased diminishing the efficacy of neuroprotective transporter substrate drugs. As neuroprotective drugs must be administered within the same time frame clinicians should be aware that increased expression can largely modulate the disposition of drugs.

Besides ABCB1, other transporters may add to the drug resistance of the brain. In a middle cerebral artery occlusion model *Abcb1* was detected mainly in newly formed capillaries in close contact with astrocytes [15], whereas in parallel a delayed 5 fold up-regulation of *Abcg2* transporter was observed. The augmentation of *Abcg2* may be attributed to formation and recovery of the endothelium of the blood vessels accompanied by cell division. A non-significant delayed increase of *ABCC5* was also seen [15].

Cerebral ischemia and reperfusion are responsible for oxidative stress and inflammation. In the first hour's post-stroke excitatory cell damage occur and in the days and weeks following they contribute to edema, inflammation and programmed cell death. Our understanding of the mechanism underlying the fine tuning and regulation of transporters is still incomplete; especially under complex circumstances, like stroke whereby several signaling cascades are involved. Several reports indicate the modulation of transporter expression under various stroke related stress stimuli from which the two main pathways hypoxia and inflammation will be considered.

The effect of hypoxic conditions on transporter activity has been widely investigated. In a hypoxia-reoxygenation model of oxidative stress induced by  $\text{H}_2\text{O}_2$  administration to primary rat brain endothelial cell cultures a biphasic

transcriptional activation of *Abcb1a* was triggered [41] where ABCB1 levels increased above basal following 6 h hypoxia and then again following 24 h of reoxygenation. ABCB1 expression returned to basal levels by 48 h. The expression level correlated well with the functional activity of the transporter. A hypoxia-inducible factor (HIF-1) binding site was identified within the promoter region of ABCB1 [41, 127], ABCC1 [128], ABCG2 [9] indicating that their expression may be regulated by hypoxia *in vivo*. And indeed, correlation was observed between mRNA expression enhancements of the transporters and HIF-1 $\alpha$ . Among others, hypoxia can also activate the transcription of genes through the PARP $\alpha$  and PPAR $\gamma$  pathways [129]. The transcriptional of ABCG2 has been reported to be regulated *via* these signaling pathways too [130, 131].

As mentioned earlier inflammation often develop after the onset of stroke. The transporter changes induced by inflammation are not straightforward and seem to be highly dependent on the model used. The transient downregulation of *Abcb1a* mRNA, and increase of drug accumulation in rat brain was reported following bacterial endotoxin exposure induced inflammation [57, 132]. Yet in another study only the activity [133] of the transporter was found to be inhibited. In contrast, ABCB1 expression was elevated in brain microvascular endothelial cells when co-cultured with activated T-lymphocytes [134]. Moreover, inhibition of ABCB1 function prevented activated T-lymphocytes mediated endothelial cell death and reduction of BBB integrity, suggesting a possible role of the transporter in neuro-inflammation. When inflammation was induced with Tumor Necrosis Factor  $\alpha$  using rat brain capillary isolates the elevation of the transporter expression followed a rapid decline of activity [135].

All in all, there are some discrepancies on the transporter changes observed following stroke, hypoxia and inflammation. It can be said that changes are highly dependent on the model used, on when and from where the samples are taken. Though several studies indicate an important role of these changes on stroke treatment further studies are required to underline this hypothesis.

## Conclusions

The present understanding of the function of transporters in stroke and stroke therapy is still in its infancy. As conventional medication to date failed mostly as a result of inefficacy novel approaches involving transporter drug interactions must also be (re)considered. Transporters may critically affect the ADME properties of drugs, the bioavailability and toxicity as well as influence the penetration of neuroprotective agents through the blood brain barrier. Several studies have shown that the barrier function of the BBB shortly after the onset of stroke is disturbed, in the core region the BBB may transiently

disintegrate, and in the penumbra region the transporter profile varies. Further comprehensive studies are needed to identify the clinical relevance of transporter activity changes in stroke, the implications of transporter interactions in the targeting of neuroprotective agents to the penumbra region.

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## References

1. Loscher, W. and H. Potschka, 2005. *Prog Neurobiol*, 76(1): p. 22.
2. Liu, X., B.J. Smith, C. Chen, E. Callegari, S.L. Becker, X. Chen, J. Cianfrogna, A.C. Doran, S.D. Doran, J.P. Gibbs, N. Hosea, J. Liu, F.R. Nelson, M.A. Szewc, and J. Van Deusen, 2006. *Drug Metab Dispos*, 34(9): p. 1443.
3. Girardin, F., 2006. *Dialogues Clin Neurosci*, 8(3): p. 311.
4. Uhr, M., A. Tontsch, C. Namendorf, S. Ripke, S. Lucae, M. Ising, T. Dose, M. Ebinger, M. Rosenhagen, M. Kohli, S. Kloiber, D. Salyakina, T. Bettecken, M. Specht, B. Putz, E.B. Binder, B. Muller-Myhsok, and F. Holsboer, 2008. *Neuron*, 57(2): p. 203.
5. Milane, A., C. Fernandez, S. Vautier, G. Bensimon, V. Meininger, and R. Farinotti, 2007. *J Neurochem*, 103(1): p. 164.
6. Regina, A., A. Koman, M. Piciotti, B. El Hafny, M.S. Center, R. Bergmann, P.O. Couraud, and F. Roux, 1998. *J Neurochem*, 71(2): p. 705.
7. Chen, X., Z.W. Zhou, C.C. Xue, X.X. Li, and S.F. Zhou, 2007. *Xenobiotica*, 37(6): p. 635.
8. Glavinas, H., P. Krajcsi, J. Cserepes, and B. Sarkadi, 2004. *Curr Drug Deliv*, 1(1): p. 27.
9. Krishnamurthy, P., D.D. Ross, T. Nakanishi, K. Bailey-Dell, S. Zhou, K.E. Mercer, B. Sarkadi, B.P. Sorrentino, and J.D. Schuetz, 2004. *J Biol Chem*, 279(23): p. 24218.
10. Sarkadi, B., L. Homolya, G. Szakacs, and A. Varadi, 2006. *Physiol Rev*, 86(4): p. 1179.
11. van Herwaarden, A.E. and A.H. Schinkel, 2006. *Trends Pharmacol Sci*, 27(1): p. 10.
12. Clayton, H., I. Titley, and M. Vivanco, 2004. *Exp Cell Res*, 297(2): p. 444-60.
13. Bronger, H., J. Konig, K. Kopplow, H.H. Steiner, R. Ahmadi, C. Herold-Mende, D. Keppler, and A.T. Nies, 2005. *Cancer Res*, 65(24): p. 11419.
14. Nies, A.T., G. Jedlitschky, J. Konig, C. Herold-Mende, H.H. Steiner, H.P. Schmitt, and D. Keppler, 2004. *Neuroscience*, 129(2): p. 349.
15. Dazert, P., Y. Suofu, M. Grube, A. Popa-Wagner, H.K. Kroemer, G. Jedlitschky, and C. Kessler, 2006. *Neuroscience*, 142(4): p. 1071.
16. Ose, A., H. Kusuhara, K. Yamatsugu, M. Kanai, M. Shibasaki, T. Fujita, A. Yamamoto, and Y. Sugiyama, 2007. *Drug Metab Dispos*.

17. Ohashi, R., Y. Kamikozawa, M. Sugiura, H. Fukuda, H. Yabuuchi, and I. Tamai, 2006. *Drug Metab Dispos*, 34(5): p. 793.
18. Polli, J.W., T.M. Baughman, J.E. Humphreys, K.H. Jordan, A.L. Mote, J.A. Salisbury, T.K. Tippin, and C.J. Serabjit-Singh, 2003. *J Pharm Sci*, 92(10): p. 2082.
19. Chen, C., E. Hanson, J.W. Watson, and J.S. Lee, 2003. *Drug Metab Dispos*, 31(3): p. 312.
20. Ak, H., B. Ay, T. Tanriverdi, G.Z. Sanus, M. Is, M. Sar, B. Oz, C. Ozkara, E. Ozyurt, and M. Uzan, 2007. *Seizure*, 16(6): p. 493.
21. Wijnholds, J., E.C. deLange, G.L. Scheffer, D.J. van den Berg, C.A. Mol, M. van der Valk, A.H. Schinkel, R.J. Scheper, D.D. Breimer, and P. Borst, 2000. *J Clin Invest*, 105(3): p. 279.
22. Kis, B., T. Isse, J.A. Snipes, L. Chen, H. Yamashita, Y. Ueta, and D.W. Busija, 2006. *J Appl Physiol*, 100(4): p. 1392.
23. Ballerini, P., P. Di Iorio, R. Ciccarelli, F. Caciagli, A. Poli, A. Beraudi, S. Buccella, I. D'Alimonte, M. D'Auro, E. Nargi, P. Patricelli, D. Visini, and U. Traversa, 2005. *Int J Immunopathol Pharmacol*, 18(2): p. 255.
24. Dallas, S., L. Schlichter, and R. Bendayan, 2004. *J Pharmacol Exp Ther*, 309(3): p. 1221.
25. Enokizono, J., H. Kusuhara, and Y. Sugiyama, 2007. *Mol Pharmacol*, 72(4): p. 967.
26. Breedveld, P., J.H. Beijnen, and J.H. Schellens, 2006. *Trends Pharmacol Sci*, 27(1): p. 17.
27. de Vries, N.A., J. Zhao, E. Kroon, T. Buckle, J.H. Beijnen, and O. van Tellingen, 2007. *Clin Cancer Res*, 13(21): p. 6440.
28. Sugiyama, D., H. Kusuhara, Y.J. Lee, and Y. Sugiyama, 2003. *Pharm Res*, 20(9): p. 1394.
29. Choudhuri, S., N.J. Cherrington, N. Li, and C.D. Klaassen, 2003. *Drug Metab Dispos*, 31(11): p. 1337.
30. Borst, P. and R.O. Elferink, 2002. *Annu Rev Biochem*, 71: p. 537.
31. Fricker, G. and D.S. Miller, 2004. *Pharmacology*, 70(4): p. 169.
32. Cooray, H.C., C.G. Blackmore, L. Maskell, and M.A. Barrand, 2002. *Neuroreport*, 13(16): p. 2059.
33. Kusuhara, H. and Y. Sugiyama, 2005. *NeuroRx*, 2(1): p. 73.
34. Sarkadi, B., E.M. Price, R.C. Boucher, U.A. Germann, and G.A. Scarborough, 1992. *J Biol Chem*, 267(7): p. 4854.
35. Lespine, A., J. Dupuy, S. Orłowski, T. Nagy, H. Glavinas, P. Krajcsi, and M. Alvinerie, 2006. *Chem Biol Interact*, 159(3): p. 169.
36. Pal, A., D. Mehn, E. Molnar, S. Gedey, P. Meszaros, T. Nagy, H. Glavinas, T. Janaky, O. von Richter, G. Bathori, L. Szente, and P. Krajcsi, 2007. *J Pharmacol Exp Ther*, 321(3): p. 1085.
37. Glavinas, H., E. Kis, A. Pal, R. Kovacs, M. Jani, E. Vagi, E. Molnar, S. Bansaghi, Z. Kele, T. Janaky, G. Bathori, O. von Richter, G.J. Koomen, and P. Krajcsi, 2007. *Drug Metab Dispos*, 35(9): p. 1533.
38. Perriere, N., S. Yousif, S. Cazaubon, N. Chaverot, F. Bourasset, S. Cisternino, X. Decleves, S. Hori, T. Terasaki, M. Deli, J.M. Scherrmann, J. Temsamani, F. Roux, and P.O. Couraud, 2007. *Brain Res*, 1150: p. 1.

39. Weksler, B.B., E.A. Subileau, N. Perriere, P. Charneau, K. Holloway, M. Leveque, H. Tricoire-Leignel, A. Nicotra, S. Bourdoulous, P. Turowski, D.K. Male, F. Roux, J. Greenwood, I.A. Romero, and P.O. Couraud, 2005. *Faseb J*, 19(13): p. 1872.
40. Cvetkovic, M., B. Leake, M.F. Fromm, G.R. Wilkinson, and R.B. Kim, 1999. *Drug Metab Dispos*, 27(8): p. 866.
41. Felix, R.A. and M.A. Barrand, 2002. *J Neurochem*, 80(1): p. 64.
42. Breedveld, P., D. Pluim, G. Cipriani, P. Wielinga, O. van Tellingen, A.H. Schinkel, and J.H. Schellens, 2005. *Cancer Res*, 65(7): p. 2577.
43. Hsiao, P., T. Bui, R.J. Ho, and J.D. Unadkat, 2007. *Drug Metab Dispos*.
44. Youdim, K.A., M.Z. Qaiser, D.J. Begley, C.A. Rice-Evans, and N.J. Abbott, 2004. *Free Radic Biol Med*, 36(5): p. 592.
45. Nies, A.T., E. Herrmann, M. Brom, and D. Keppler, 2008. *Naunyn Schmiedebergs Arch Pharmacol*, 376(6): p. 449.
46. Hollo, Z., L. Homolya, C.W. Davis, and B. Sarkadi, 1994. *Biochim Biophys Acta*, 1191(2): p. 384.
47. Ozvegy, C., A. Varadi, and B. Sarkadi, 2002. *J Biol Chem*, 277(50): p. 47980-90.
48. Robey, R.W., K. Steadman, O. Polgar, K. Morisaki, M. Blayney, P. Mistry, and S.E. Bates, 2004. *Cancer Res*, 64(4): p. 1242.
49. Yamagata, T., H. Kusuhara, M. Morishita, K. Takayama, H. Benameur, and Y. Sugiyama, 2007. *J Control Release*, 124(1-2): p. 1.
50. Tsuji, A., T. Terasaki, Y. Takabatake, Y. Tenda, I. Tamai, T. Yamashima, S. Moritani, T. Tsuruo, and J. Yamashita, 1992. *Life Sci*, 51(18): p. 1427.
51. Jonker, J.W., E. Wagenaar, L. van Deemter, R. Gottschlich, H.M. Bender, J. Dasenbrock, and A.H. Schinkel, 1999. *Br J Pharmacol*, 127(1): p. 43.
52. Reichel, A., D.J. Begley, and N.J. Abbott, 2003. *Methods Mol Med*, 89: p. 307.
53. Miller, D.S., S.N. Nobmann, H. Gutmann, M. Toeroek, J. Drewe, and G. Fricker, 2000. *Mol Pharmacol*, 58(6): p. 1357.
54. Fricker, G., S. Nobmann, and D.S. Miller, 2002. *Br J Pharmacol*, 135(5): p. 1308.
55. Schinkel, A.H., 1998. *Int J Clin Pharmacol Ther*, 36(1): p. 9.
56. Cisternino, S., C. Mercier, F. Bourasset, F. Roux, and J.M. Scherrmann, 2004. *Cancer Res*, 64(9): p. 3296.
57. Goralski, K.B., G. Hartmann, M. Piquette-Miller, and K.W. Renton, 2003. *Br J Pharmacol*, 139(1): p. 35.
58. Lankas, G.R., M.E. Cartwright, and D. Umbenhauer, 1997. *Toxicol Appl Pharmacol*, 143(2): p. 357.
59. Leggas, M., M. Adachi, G.L. Scheffer, D. Sun, P. Wielinga, G. Du, K.E. Mercer, Y. Zhuang, J.C. Panetta, B. Johnston, R.J. Scheper, C.F. Stewart, and J.D. Schuetz, 2004. *Mol Cell Biol*, 24(17): p. 7612.
60. Hosoya, K., H. Asaba, and T. Terasaki, 2000. *Life Sci*, 67(22): p. 2699.
61. Terasaki, T. and S. Ohtsuki, 2005. *NeuroRx*, 2(1): p. 63.
62. Yu, X.Y., S.G. Lin, X. Chen, Z.W. Zhou, J. Liang, W. Duan, B. Chowbay, J.Y. Wen, E. Chan, J. Cao, C.G. Li, and S.F. Zhou, 2007. *Curr Drug Metab*, 8(4): p. 365.
63. Luurtsema, G., C.F. Molthoff, R.C. Schuit, A.D. Windhorst, A.A. Lammertsma, and E.J. Franssen, 2005. *Nucl Med Biol*, 32(1): p. 87.
64. Elsinga, P.H., N.H. Hendrikse, J. Bart, W. Vaalburg, and A. van Waarde, 2004. *Curr Pharm Des*, 10(13): p. 1493.

65. Hendrikse, N.H., 2000. *Curr Pharm Des*, 6(16): p. 1653.
66. Rao, V.V., J.L. Dahlheimer, M.E. Bardgett, A.Z. Snyder, R.A. Finch, A.C. Sartorelli, and D. Piwnica-Worms, 1999. *Proc Natl Acad Sci U S A*, 96(7): p. 3900.
67. Green, A.R., 2007. *Br J Pharmacol*.
68. Shuaib, A., K.R. Lees, P. Lyden, J. Grotta, A. Davalos, S.M. Davis, H.C. Diener, T. Ashwood, W.W. Wasiewski, and U. Emeribe, 2007. *N Engl J Med*, 357(6): p. 562.
69. Gogas, K.R., 2006. *Curr Opin Pharmacol*, 6(1): p. 68.
70. Negishi, H., J.W. Xu, K. Ikeda, M. Njelekela, Y. Nara, and Y. Yamori, 2004. *J Nutr*, 134(1): p. 38.
71. Arts, I.C. and P.C. Hollman, 2005. *Am J Clin Nutr*, 81(1 Suppl): p. 317S-325S.
72. Davis, S., K. Lees, and G. Donnan, 2006. *Int J Clin Pract*, 60(4): p. 399.
73. Ringleb, P.A., 2006. *Stroke*, 37(2): p. 312.
74. Wang, C.X. and A. Shuaib, 2007. *Drugs Aging*, 24(7): p. 537.
75. Schinkel, A.H., 2001. *Adv Exp Med Biol*, 500: p. 365.
76. Wadelius, M., K. Sorlin, O. Wallerman, J. Karlsson, Q.Y. Yue, P.K. Magnusson, C. Wadelius, and H. Melhus, 2004. *Pharmacogenomics J*, 4(1): p. 40.
77. Zhou, S., E. Chan, S.Q. Pan, M. Huang, and E.J. Lee, 2004. *J Psychopharmacol*, 18(2): p. 262.
78. Pal, D. and A.K. Mitra, 2006. *Life Sci*, 78(18): p. 2131.
79. Finch, C.K., C.R. Chrisman, A.M. Baciewicz, and T.H. Self, 2002. *Arch Intern Med*, 162(9): p. 985.
80. Dorani, H., K.M. Schutzer, T.C. Sarich, U. Wall, U. Logren, L. Ohlsson, and U.G. Eriksson, 2007. *Eur J Clin Pharmacol*, 63(6): p. 571.
81. Eriksson, U.G., H. Dorani, J. Karlsson, H. Fritsch, K.J. Hoffmann, L. Olsson, T.C. Sarich, U. Wall, and K.M. Schutzer, 2006. *Drug Metab Dispos*, 34(5): p. 775.
82. Leonardi-Bee, J., P.M. Bath, M.G. Bousser, A. Davalos, H.C. Diener, B. Guiraud-Chaumeil, J. Sivenius, F. Yatsu, and M.E. Dewey, 2005. *Stroke*, 36(1): p. 162.
83. Hill, B.T., 1990. *Cancer Treat Rev*, 17(2-3): p. 197.
84. Hosoi, E., M. Hirose, S. Hamano, M. Morimoto, and Y. Kuroda, 1998. *Int J Oncol*, 13(2): p. 343.
85. Khan, E.U., A. Reichel, D.J. Begley, S.J. Roffey, S.G. Jezequel, and N.J. Abbott, 1998. *Int J Clin Pharmacol Ther*, 36(2): p. 84.
86. Zhang, Y., A. Gupta, H. Wang, L. Zhou, R.R. Vethanayagam, J.D. Unadkat, and Q. Mao, 2005. *Pharm Res*, 22(12): p. 2023.
87. Verstuyft, C., S. Strabach, H. El-Morabet, R. Kerb, U. Brinkmann, L. Dubert, P. Jaillon, C. Funck-Brentano, G. Trugnan, and L. Becquemont, 2003. *Clin Pharmacol Ther*, 73(1): p. 51.
88. Mehta, S.L., N. Manhas, and R. Raghbir, 2007. *Brain Res Rev*, 54(1): p. 34.
89. Abraham, I., C.L. Wolf, and K.E. Sampson, 1993. *Cancer Chemother Pharmacol*, 32(2): p. 116.
90. Mizuno, N., T. Takahashi, H. Kusuhara, J.D. Schuetz, T. Niwa, and Y. Sugiyama, 2007. *Drug Metab Dispos*, 35(11): p. 2045.
91. Hishida, A., 2007. *Clin Exp Nephrol*, 11(4): p. 292.
92. Werling, L.L., E.C. Lauterbach, and U. Calef, 2007. *Neurologist*, 13(5): p. 272.

93. Arellano, C., C. Philibert, C. Vachoux, J. Woodley, and G. Houin, 2007. *J Pharm Pharm Sci*, 10(1): p. 26.
94. Marier, J.F., J.L. Deschenes, A. Hage, E. Seliniotakis, A. Gritsas, T. Flarakos, F. Beaudry, and P. Vachon, 2005. *Life Sci*, 77(23): p. 2911.
95. Uhr, M., C. Namendorf, M.T. Grauer, M. Rosenhagen, and M. Ebinger, 2004. *J Psychopharmacol*, 18(4): p. 509.
96. Weng, Y.C. and J. Kriz, 2007. *Exp Neurol*, 204(1): p. 433.
97. Siniscalchi, A., C. Zona, G. Sancesario, E. D'Angelo, Y.C. Zeng, N.B. Mercuri, and G. Bernardi, 1999. *Synapse*, 32(3): p. 147.
98. Paquet-Durand, F., A. Gierse, and G. Bicker, 2006. *Brain Res*, 1124(1): p. 45.
99. Ikeda, K., H. Negishi, and Y. Yamori, 2003. *Toxicology*, 189(1-2): p. 55.
100. Huang, S.S., M.C. Tsai, C.L. Chih, L.M. Hung, and S.K. Tsai, 2001. *Life Sci*, 69(9): p. 1057.
101. Sinha, K., G. Chaudhary, and Y.K. Gupta, 2002. *Life Sci*, 71(6): p. 655.
102. Dore, S., 2005. *Neurosignals*, 14(1-2): p. 61.
103. Zhu, A., X. Wang, and Z. Guo, 2001. *Nucl Med Biol*, 28(6): p. 735.
104. Schutte, M.E., A.P. Freidig, J.J. van de Sandt, G.M. Alink, I.M. Rietjens, and J.P. Groten, 2006. *Toxicol Appl Pharmacol*, 217(2): p. 204.
105. Hong, J., J.D. Lambert, S.H. Lee, P.J. Sinko, and C.S. Yang, 2003. *Biochem Biophys Res Commun*, 310(1): p. 222.
106. Wortelboer, H.M., M. Usta, A.E. van der Velde, M.G. Boersma, B. Spenkelink, J.J. van Zanden, I.M. Rietjens, P.J. van Bladeren, and N.H. Cnubben, 2003. *Chem Res Toxicol*, 16(12): p. 1642.
107. Zhang, X.M., J.T. Li, M. Zhu, X.L. Wu, P. Gao, P. Zhou, and Y.P. Wang, 2004. *Zhonghua Liu Xing Bing Xue Za Zhi*, 25(11): p. 978.
108. de Boer, A.G. and P.J. Gaillard, 2007. *Annu Rev Pharmacol Toxicol*, 47: p. 323.
109. Ellis-Behnke, R.G., L.A. Teather, G.E. Schneider, and K.F. So, 2007. *Curr Pharm Des*, 13(24): p. 2519.
110. Singh, S. and H.S. Nalwa, 2007. *J Nanosci Nanotechnol*, 7(9): p. 3048.
111. Brandt, C., K. Bethmann, A.M. Gastens, and W. Loscher, 2006. *Neurobiol Dis*, 24(1): p. 202.
112. van Vliet, E.A., R. van Schaik, P.M. Edelbroek, S. Redeker, E. Aronica, W.J. Wadman, N. Marchi, A. Vezzani, and J.A. Gorter, 2006. *Epilepsia*, 47(4): p. 672.
113. van Vliet, E.A., R. van Schaik, P.M. Edelbroek, R.A. Voskuyl, S. Redeker, E. Aronica, W.J. Wadman, and J.A. Gorter, 2007. *J Pharmacol Exp Ther*, 322(1): p. 141.
114. El Ela, A.A., S. Hartter, U. Schmitt, C. Hiemke, H. Spahn-Langguth, and P. Langguth, 2004. *J Pharm Pharmacol*, 56(8): p. 967.
115. Anderson, B.D., M.J. May, S. Jordan, L. Song, M.J. Roberts, and M. Leggas, 2006. *Drug Metab Dispos*, 34(4): p. 653.
116. Shaik, N., N. Giri, G. Pan, and W.F. Elmquist, 2007. *Drug Metab Dispos*, 35(11): p. 2076.
117. Hubensack, M., C. Muller, P. Hochehl, S. Fellner, T. Spruss, G. Bernhardt, and A. Buschauer, 2007. *J Cancer Res Clin Oncol*.
118. Kemper, E.M., C. Cleypool, W. Boogerd, J.H. Beijnen, and O. van Tellingen, 2004. *Cancer Chemother Pharmacol*, 53(2): p. 173.

119. Kemper, E.M., W. Boogerd, I. Thuis, J.H. Beijnen, and O. van Tellingen, 2004. *Cancer Treat Rev*, 30(5): p. 415.
120. Spudich, A., E. Kilic, H. Xing, U. Kilic, K.M. Rentsch, H. Wunderli-Allenspach, C.L. Bassetti, and D.M. Hermann, 2006. *Nat Neurosci*, 9(4): p. 487.
121. Lazarowski, A., L. Czornyj, F. Lubienieki, E. Girardi, S. Vazquez, and C. D'Giano, 2007. *Epilepsia*, 48 Suppl 5: p. 140.
122. Cho, C.W., Y. Liu, W.N. Cobb, T.K. Henthorn, K. Lillehei, U. Christians, and K.Y. Ng, 2002. *Pharm Res*, 19(8): p. 1123.
123. Liu, Y., K. Lillehei, W.N. Cobb, U. Christians, and K.Y. Ng, 2001. *Biochem Biophys Res Commun*, 289(1): p. 62.
124. Abbruscato, T.J. and T.P. Davis, 1999. *J Pharmacol Exp Ther*, 289(2): p. 668.
125. Ramos, A.J., A. Lazarowski, M.J. Villar, and A. Brusco, 2004. *Cell Mol Neurobiol*, 24(1): p. 101.
126. Lazarowski, A., L. Caltana, A. Merelli, M.D. Rubio, A.J. Ramos, and A. Brusco, 2007. *J Neurol Sci*, 258(1-2): p. 84.
127. Comerford, K.M., T.J. Wallace, J. Karhausen, N.A. Louis, M.C. Montalto, and S.P. Colgan, 2002. *Cancer Res*, 62(12): p. 3387.
128. Zhu, H., X.P. Chen, S.F. Luo, J. Guan, W.G. Zhang, B.X. Zhang, and H.P. Wang, 2005. *Zhonghua Wai Ke Za Zhi*, 43(5): p. 277.
129. Sundararajan, S., Q. Jiang, M. Heneka, and G. Landreth, 2006. *Neurochem Int*, 49(2): p. 136.
130. Szatmari, I., E. Rajnavolgyi, and L. Nagy, 2006. *Ann N Y Acad Sci*, 1088: p. 207.
131. Hirai, T., Y. Fukui, and K. Motojima, 2007. *Biol Pharm Bull*, 30(11): p. 2185.
132. Wang, J.H., D.A. Scollard, S. Teng, R.M. Reilly, and M. Piquette-Miller, 2005. *J Nucl Med*, 46(9): p. 1537.
133. Hartz, A.M., B. Bauer, G. Fricker, and D.S. Miller, 2004. *Mol Pharmacol*, 66(3): p. 387.
134. Tan, K.H., W.M. Purcell, S.J. Heales, J.D. McLeod, and R.D. Hurst, 2002. *Neuroreport*, 13(18): p. 2593.
135. Bauer, B., A.M. Hartz, and D.S. Miller, 2007. *Mol Pharmacol*, 71(3): p. 667.